FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)
344-P-30-USA	10/069836

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/20017	20 July, 2000	20 July, 2000

TITLE OF INVENTION

IMPROVED DIAGNOSTIC METHOD FOR DETECTING DYSPLASTIC EPITHELIAL TISSUE

APPLICANT(S) FOR DO/EO/US

Douglas D. Burkett

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☑ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☑ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☑ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☑ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☑ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☑ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☑ Certificate of Mailing by Express Mail
23. ☑ Other items or information:

Copy of previously submitted Assignment document filed in the International Application.

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/069836	PCT/US00/20017	344-P-30-USA

24. The following fees are submitted:.		CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :			
☑ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1040.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$890.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$740.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$710.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =		**$1,040.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☑ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		**$130.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	2 - 20 =	0	x $18.00	**$0.00**	
Independent claims	2 - 3 =	0	x $84.00	**$0.00**	
Multiple Dependent Claims **(check if applicable).**			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,170.00**	
☑ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$585.00**	
SUBTOTAL =				**$585.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$585.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$585.00**	
				Amount to be: refunded	$
				charged	$

a. ☐ A check in the amount of _____ to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☐ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. _____ A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

David G. Duckworth
NAME

39,516
REGISTRATION NUMBER

February 26, 2002
DATE

IMPROVED DIAGNOSTIC METHOD FOR DETECTING DYSPLASTIC EPITHELIAL TISSUE

This invention relates to an improved diagnostic method for *in vivo* detection of dysplastic epithelial tissue.

In a more particular respect, the invention is an improved diagnostic method for detecting and/or delineating cancerous or precancerous epithelial tissue, with a reduced rate of false positives.

According to another aspect of the invention, the false positive rate of diagnostic methods that involve topical application of a dye that selectively stains cancerous and precancerous epithelial tissue is markedly reduced.

These and other, further and more specific aspects of the invention will be apparent to those skilled in the art from the following description thereof.

It is known that various cationic supravital dyes have the capability of selectively staining cancerous and precancerous cells of epithelial tissue, as well as cells

that are abnormal due to dysplasia, hyperplasia, tumorigenesis and other active surface lesions.. For example, such dyes are disclosed in U.S. Patents Nos. 4,321,251 to Mashberg, 5,372,801 to Tucci, *et al.*, 5,882,627 to Pomerantz, and the pending International Application of Bernal *et al.*, PCT/US00/05387. Also, see Chenz, Chinese Journal of Stomatology (27:44-47)(1992) and Filurin, Stomatologiia (Russian) (72:44-47)(1993). Other dyes that are similarly useful include rhodamine, alcian blue, malachite green, phenosafranin, acriflavine, pyronine Y, toluylene blue, and brilliant green. "Non-dye" compounds that are similarly useful include peonidin, oxythiamine, tiemonium iodide, elliptinium acetate and furazolium chloride.

The mechanism of such selective staining has been shown to involve absorption or entry of the marking agent molecule into the mitochondria of the cancerous or precancerous epithelial cells. This selective staining of the mitochondria of cancerous tissue is apparently due to the higher electrical potential (negative charge on the inside of the membrane of cancerous mitochondrial cells as compared to normal cells.

Although the mitochondrial marking agent also temporarily stains nearby non-cancerous tissue, it is released much more quickly from the normal tissue than from the mitochondria of the cancerous tissue. Thus the diagnosis of cancer is based on the continued retention of the dye in the cancerous tissue after it is autogenously released from the normal tissue. Proper selection of the elapsed time between application of the dye and the diagnostic observation of the tissue, permits the diagnostician to detect and selectively delineate cancerous or precancerous tissue sites on normal epithelial surfaces. This procedure permits identification of cancerous and potential cancerous sites with a high degree of accuracy, i.e., with a very low incidence of false negatives. However, because of differences in the tissues between patients and other variables such as skill of the diagnostician, etc., this diagnostic technique may also yield false positive results.

While false positives are much preferred over false negative results, it would, nevertheless, be highly desirable to reduce the rate of false positives, to avoid or reduce the necessity for invasive confirmatory testing

and to avoid unnecessarily upsetting the patient.

To attempt to reduce the rate of false positives, it has been proposed to repeat the procedure after approximately two weeks, which gives time for healing of non-cancerous lesions or wounds which apparently tend to accumulate and retain the dye longer than normal tissue, even though they are not cancerous or precancerous. Of course, this repetition does prevent a number of false positives. However, the potential still remains for false positive due to other causes.

The temporary, less pronounced tendency of these dyes to stain normal tissue is due to binding of the dye with components of the extracellular matrix ("ECM") of epithelial tissue. Whereas the dye actually enters the mitochondria of cancerous and precancerous cells, it is only temporarily bound to components of the ECM, particularly to fibronectin.

Temporary binding of cationic dyes and other mitochondrial marking agents to ECM components may be due to one or more of a variety of mechanisms. Thus the mitochondrial marking agents may be temporarily bound to

negatively charged ECM proteins by electrostatic attraction. Furthermore, hydrophobic interactions may take place between the ECM proteins and heterocyclic portions of the marking agent which exclude water. Other non-specific binding may occur by binding of various portions of the marking agent to ECM proteins that bind neutral charges. Such temporary binding of mitochondrial marking agents to ECM proteins can occur even outside of the tight junctions between epithelial cells, e.g., on the surface of the epithelium, as well as between and beneath cancerous cells.

The undesired temporary binding of mitochondrial marking agents to ECM proteins can be largely prevented by pretreating the area of the epithelium to which the marking agent is to be applied with a non-toxic amphiphilic protein. The amphiphilic protein enters the various binding mechanisms to the ECM proteins, thus temporarily disabling them from binding the mitochondial marking agent when it is later applied. Such pretreatment of the epithelium with amphiphilic protein markedly reduces the occurrence of false positive reactions engendered by temporary binding of the mitochondrial marking agent to ECM proteins and the

consequent appearance of "stained" areas on the normal epithelium which might be mistaken for cancerous or precancerous tissue.

The exact nature of the amphiphilic protein to be applied as a pretreatment is not highly critical. All mucopolysaccharides are amphiphilic. However, for ease of handling and application, it is presently preferred to employ albumins (soluble in water) or globulins (soluble in dilute salt solutions). For example, serum albumin and milk proteins, such as casein, are effectively employed. Gluten proteins, such as wheat albumins and prolamins (soluble in aqueous alcohol) and glutenins (soluble in dilute acids and bases, detergents or reducing agents) are also effectively employed.

The following examples illustrate the presently preferred practice of the invention. Those skilled in the art will understand and appreciate modifications of this procedure that can be made without departing from the basic concept of the invention. Consequently, these examples are not to taken as limiting the scope of the invention, which is defined only by the appended claim.

EXAMPLE 1

Preparation of Pre-Treatment Composition

The following amphiphilic protein pre-treatment composition is prepared:

Component	Weight %
Serum albumin	30
Sterile water	68.5
Flavor (IFF Raspberry IC563457)	.5
Preservative (sodium benzoate)	1.0

EXAMPLE 2

Preparation of TBO Stain Composition

A toluidine blue O ("TBO") stain compositio5 is prepared, having the following composition

Component	Weight %
TBO	1.00
Flavor (IFF Raspberry IC563457)	.20
Buffering Agent (sodium acetate trihydrate)	2.45
Preservative (hydrogen peroxide 30%)	.41
Acetic acid	4.61
Ethyl alcohol	7.48
Water	83.85

EXAMPLE 3

Preparation of Pre-rinse and Post-rinse Solutions

Pre-rinse and post-rinse solutions of 1 wt% acetic acid in purified water, sodium benzoate preservative and raspberry flavor are prepared.

EXAMPLE 4

Clinical Protocol

The patient is draped with a bib to protect clothing. Expectoration is expected, so the patient is provided with a 10-oz. cup, which can be disposed of in an infectious waste container or the contents can be poured directly into the center drain of a sink to avoid staining the sink. Environmental surfaces or objects which might be stained are draped or removed from the area.

A visual oral cancer examination is conducted, without using any instruments which might cause nicks or cuts of soft tissues. Notations are made of the appearance of soft tissues and teeth.

The patient rinses the oral cavity with approximately 15 ml of the of the pre-rinse solution for approximately 20 seconds and expectorates, to remove excess saliva and provide a consistent oral environment. This step is then repeated with additional pre-rinse solution.

The patient then rinses and gargles with water for approximately 20 seconds and expectorates.

The patient then rinses and gargles with approximately 50 ml of the protein pretreatment composition for approximately 30 seconds and expectorates. This step is then repeated, except that the patient retains the protein pretreatment composition within the mouth for approximately two minutes, then expectorates.

The patient then rinses and gargles with 30 ml. of the TBO solution for one minute and expectorates.

The patient then rinses with 15 ml of the post rinse solution and expectorates. This step is then repeated.

The patient then rinses and gargles with water for 20 seconds and expectorates. This step is then repeated.

Visual observations of the oral cavity are then made, using appropriate soft-tissue examination techniques, including retraction, well-balanced lighting and magnification, if necessary. The location, size, morphology, color and surface characteristics of suspect lesions, that have retained blue coloration are made and recorded.

Specimens of any tissues that have retained blue coloration are obtained and subjected to normal cancer-detection histological procedures. No "false positives" specimens are noted.

EXAMPLE 5

Use of Other Proteins

The procedures of Examples 1-4 are repeated except that the protein pre-treatment solution of Example 1 consists of globulins, casein, gluten albumin, wheat prolamin and glutenins in suitable pharmacologically acceptable solvents, with suitable flavorings.

Equivalent results are obtained.

EXAMPLE 6

Use of Other Mitochondrial Marking Dyes

The procedures of Examples 1-5 are repeated except that the staining dyes employed are Azure B, Azure C, Brilliant Cresyl Blue, Rhodamine, Alcian Blue, Malachite Green, Phenosafranin, Acriflavine, Pyronine Y, Toluylene Blue, Brilliant Green, Peonidin, Oxythiamine, tiemonium iodide, elliptinium acetate and furazolium chloride. Equivalent results are obtained.

Having described my invention in such terms as to enable those skilled in the art to understand and practice it and, having identified the presently preferred embodiments thereof, I CLAIM:

1. In a diagnostic method for detecting dysplastic epitheleal tissue, which includes the step of

topically applying a mitochondrial marking agent to the locus of suspect tissue which selectively stains cancerous and precancerous cells,

the method of decreasing the rate of false positives of said method comprising inhibiting the marking of extracellular matrix components by said stain, by applying a protein to said locus, prior to application of said stain.

2. The use of an amphiphilic protein to pretreat epithelial tissue before application of a mitochondrial marking agent for detecting cancerous or precancerous tissue, to bind ECM proteins and reduce the likelihood of a false positive indication.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(81) Designated States *(national)*: AT, AU, BR, CH, CN, CZ, HU, IL, IN, JP, KR, MX, NO, NZ, PL, RO, SG, SK, TR, UA, US, ZA.

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Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/07693 A1

(54) Title: IMPROVED DIAGNOSTIC METHOD FOR DETECTING DYSPLASTIC EPITHELIAL TISSUE

(57) Abstract: A method of intraoral toluidine blue staining is disclosed where the pre-rinse composition contains amphiphilic protein, such as albumin, which binds to extracellular matrix components such as fibronectin. In this way, the staining is more specific to precancerous and cancerous cells.

Docket No.
344-P-30-USA

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
IMPROVED DIAGNOSTIC METHOD FOR DETECTING DYSPLASTIC EPITHELIAL TISSUE

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on **20 July, 2000** as United States Application No. or PCT International Application Number **PCT/US00/20017**

and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

PCT/US00/20017	**20 July, 2000**	**Pending**
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

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Citizenship **USA**
Post Office Address **Same as above**

Full name of second inventor, if any
Second inventor's signature Date
Residence
Citizenship
Post Office Address

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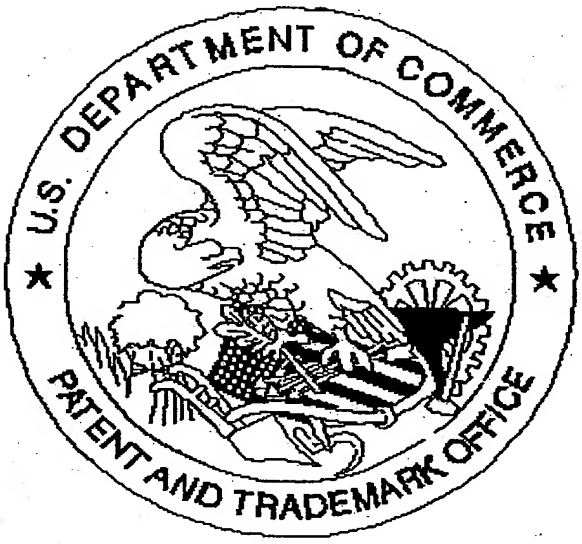

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